ISSN 0006-2979, Biochemistry (Moscow), 2009, Vol. 74, No. 1, pp. 22-28. © Pleiades Publishing, Ltd., 2009.
Published in Russian in Biokhimiya, 2009, Vol. 74, No. 1, pp. 30-38.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM08-031, December 14, 2008.

Identification of a Novel Gene, *MSAG*, Regulated by High Levels of Glucose and Insulin

Xiao Yan Cui, Jin Xin Chen, Bing Wen Liu, Li Ying Xiao, and Ding Zhi Fang*

Department of Biochemistry and Molecular Biology, State Key Laboratory of Oral Diseases, West China Medical Center, Sichuan University, Chengdu 610041, P. R. China; fax: 86-28-8550-3204; E-mail: dzfang@scu.edu.cn; fangdz@gmail.com

Received January 30, 2008
Revision received June 8, 2008

Abstract—Identification and characterization of novel genes involved in derangement of metabolisms of glucose and triglycerides are important in understanding the development of metabolic syndrome (MS) and atherosclerosis. Model rats with certain phenotypes of MS were fed a high-carbohydrate diet. The rat hepatic subtracted cDNA libraries were constructed and screened. A novel cDNA of full length was identified by screening of a human hepatic cDNA library with a mixture of probes of the differentially expressed fragments from the rat hepatic subtracted cDNA libraries. The corresponding gene of the cDNA was temporarily named metabolic syndrome-associated gene (*MSAG*). The predicted protein encoded by *MSAG* contains 110 amino acids and has a theoretical molecular weight of 11667.04 and an isoelectric point of 4.91. Compared with the housekeeping gene of β-actin, *MSAG* had low transcription activity. However, the mRNA level of *MSAG* in HepG2 cells, a human hepatoma cell line, was significantly increased by glucose and decreased by insulin concentrations higher than physiological levels. These results suggest that *MSAG* may be involved in the metabolism and/or its regulation of glucose, the functioning of insulin under non-physiological conditions, and further in the development of metabolic syndrome.

DOI: 10.1134/S0006297909010039

Key words: differentially expressed genes, cDNA library, screening, glucose, metabolic syndrome-associated gene, HepG2 cells, real-time quantitative PCR

Metabolic syndrome (MS), first described by Reaven [1], is comprised of obesity, hypertension, impaired glucose tolerance or diabetes, hyperinsulinemia, and dyslipidemia characterized by elevated triglyceride and low HDL (high density lipoprotein) concentrations. Several new features have been added to the syndrome over time, including pro-inflammatory states, microalbuminuria, and hypercoagulability [2-4]. This syndrome has been found to be significantly associated with coronary heart disease, stroke, and type 2 diabetes mellitus [5-7].

Liver has important roles in the metabolisms of glucose and lipids, coagulation, and fibrinolysis. The production of glucose by liver is an essential process that maintains systemic normal levels of glucose [8]. As the principal organ of lipogenesis, liver is responsible for the production of triglycerides from excess dietary carbohydrate [9] and degradation of apoB-100-containing lipoproteins [10]. The majority of factors required for coagulation as well as fibrinolytic cascades are generated and secreted into the bloodstream by liver, which plays important roles in the development of hypercoagulable states [11]. Therefore, liver plays key roles in the development of MS.

The pathogenesis of MS has multiple origins and has been found to be the result of complex interactions between genetic and environmental factors [12]. The prevalence of MS varies very greatly, from 7 to 84% [13, 14]. These differences can be explained by the criteria involved in MS, age, sex, and study populations. In addition, genetic differences must be considered because variations of MS prevalence indicate that some people have a genetic predisposition making them more susceptible to MS although they are living in the same environments [15, 16]. Mutations and polymorphisms in some genes may be the determinants of this genetic predisposition. In fact, a series of genes have been identified to be associat-

Abbreviations: APTT, activated partial thromboplastin time; DAG, diacylglycerol; HDL, high density lipoprotein; HRP, horseradish peroxidase; MS, metabolic syndrome; *MSAG*, metabolic syndrome-associated gene; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin time; SSH, suppression subtractive hybridization.

* To whom correspondence should be addressed.

ed with insulin resistance, adipocyte abnormality, hypertension, and lipid abnormalities, which have been shown to be associated with MS [12, 17]. However, functions of these genes cannot explain all the pathophysiology of MS. More studies are needed to identify the unknown genetic factors that interact with environmental factors to produce the syndrome.

In the present study, we introduced a rat model having some features of MS and constructed and screened rat hepatic subtracted cDNA libraries. A novel cDNA of full length was identified by screening of a human hepatic cDNA library with the mixture as probes of the differentially expressed fragments from the rat hepatic subtracted cDNA libraries. We temporarily name this novel gene as metabolic syndrome-associated gene, *MSAG*. Some characteristics of this novel gene have been determined in HepG2 cells.

MATERIALS AND METHODS

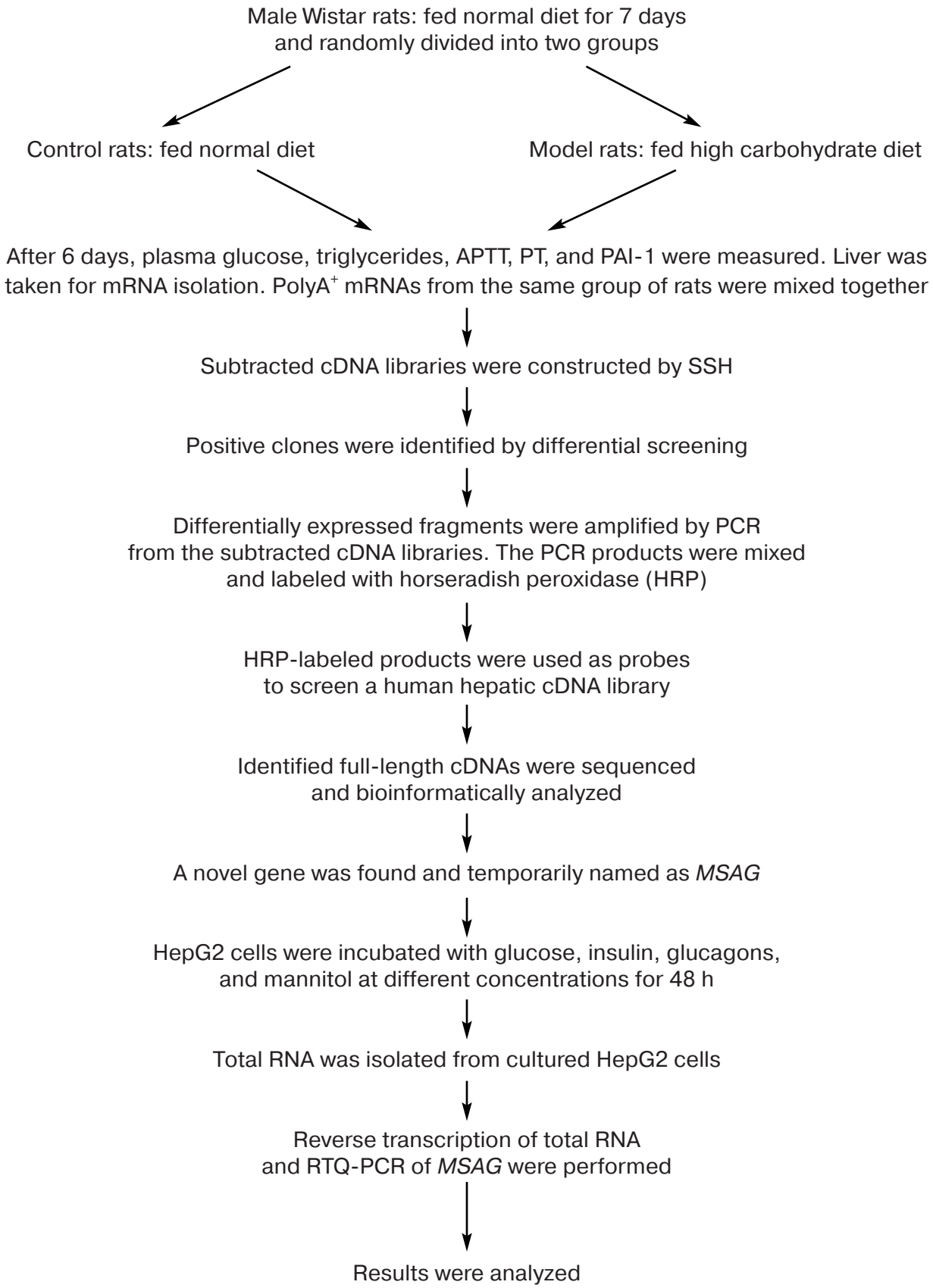
The flow diagram of this experiment is shown in the Scheme. Male Wistar rats of about 250 g were purchased from Sichuan University Animal Center. After arriving, all the animals were fed normal commercial diet containing 62% carbohydrate, 17% protein, and 21% fat of the total calories. After 7 days, the rats were randomly divided into two groups. The control rats were still fed the normal diet. The model rats received a prepared high carbohydrate diet containing 80% carbohydrate, 15% protein, and 5% fat of the total calories for six days. The levels of plasma glucose, triglycerides, and plasminogen activator inhibitor-1 (PAI-1) as well as activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured as described previously [18].

Total RNA and polyA$^+$ mRNA were isolated and purified from model or control rats using the EZNA® Total RNA Maxiprep kit (Omega Biotek, USA) and Oligotex Midi mRNA kit (Qiagen, USA), respectively. PolyA$^+$ mRNAs from the same group of rats were mixed together. Reverse transcription of polyA$^+$ mRNA was performed with SMART PCR cDNA Synthesis kit (Clontech, USA). The subtracted cDNA libraries were constructed by suppression subtractive hybridization (SSH) using PCR-Select cDNA Subtraction kit (Clontech). After two hybridizations, two suppression PCR amplifications were performed. The subtracted PCR products were cloned into the pMD18-T vector (TaKaRa Biotech, China). Positive clones were identified by differential screening of the forward- and reverse-subtracted cDNA libraries.

The differentially expressed fragments were amplified by PCR from the positive cDNA clones of the subtracted cDNA libraries. The PCR products were cleaned, mixed, and labeled with horseradish peroxidase (HRP) using North2South® Direct HRP Labeling and Detection kit (Pierce, USA). The HRP-labeled products were then used as probes to screen a human hepatic cDNA library (Clontech). After the first, second, and third screening, transduction of a positive λTripIEx lysate into *Escherichia coli* strain BM25.8 promoted circularization of pTripIEx. The positive circularized plasmids were identified by double enzyme digestion. The identified full-length cDNAs were sequenced. From the full-length cDNA sequences, deduced partial protein sequences were blasted against the GenBank database using the BLAST program. As the result, a novel gene was found and temporarily named as metabolic syndrome-associated gene, *MSAG*. Further analysis of the predicted protein was performed by using ExPASy search programs (http://cn.expasy.org/tools). For alignment of different mammals, amino acid sequences of MSAG of human (GenBank accession number, NM_173473.2), mouse (BC025117), Bornean orangutan (CR857550), rhesus monkey (XM_001106406), dog (XM_856703), pig (AK238981), and cow (BC114889) were retrieved from the Nucleotide Collection (nr/nt) Database using the tblastn program (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). *MSAG* gene sequences of rat and horse were retrieved from the UCSC Genome Browser Database (http://genome.ucsc.edu/) using the BLAT tool (http://genome.ucsc.edu/cgi-bin/hgBlat). These gene sequences were translated into amino acid sequences using the DNAStar tool. Amino acid sequences were aligned using the software ClustalX.

HepG2 cells, a human hepatoma cell line, were maintained in DMEM medium (5.6 mM glucose) (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 mM L-glutamine (Amresco, USA) at 37°C and 5% CO_2. After being grown to 90% confluence, the cells were starved for 24 h in a serum-free DMEM and then incubated with glucose- and serum-free medium with 0.5% bovine serum albumin (BSA) (Roche Applied Science, Germany) at different concentrations of glucose (2.8, 5.6, 11.1, 22, and 33.3 mM) or serum-free medium with 0.5% BSA at different concentrations of insulin (0, 0.1, 1, 10, 100, and 1000 nM) or glucagon (0, 0.01, 0.1, 1, 10, 100, and 1000 nM), respectively, for 48 h. Mannitol was used as the control of osmotic pressure by adding different concentrations of mannitol (0, 2.8, 8.3, 19.2, 30.5 mM) to the medium.

Total RNA was isolated from cultured HepG2 cells and the possibly remaining DNA was digested by RNase-free DNase I (TaKaRa Biotech). Oligo(dT) primers and ReverTra Ace reverse transcriptase (Toyobo, Japan) were used to perform reverse transcription of total RNA. Real-time quantitative PCR (RTQ-PCR) was performed using an ABI 7300 Real-Time PCR System and Sequence Detection Software (version 1.3.1) (Applied Biosystems, USA) by the following cycle parameters: 1 min at 95°C,



Scheme of the present study

followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. All data were normalized to *β-actin* expression. For each sample, RTQ-PCR was conducted in triplicate with a reaction volume of 25 μl. The following primers and probes synthesized by TaKaRa were used for the PCR. For *MSAG*: forward primer, 5′-GGGTCAGTGGAAGTTCTGTCAC-3′; reverse primer, 5′-TCTCTCAGAGCCATCTTCTAACATC-3′; oligonucleotide probe, 5′-FAM-CTGGTTTCAGTGTCTCAGACCTTGCCCTAMRA-3′. For *β-actin*: forward primer, 5′-ACCCTGAAGTACCCCATCGAG-3′; reverse primer, 5′-ACATGATCTGGGTCATCTTCTCG-3′; oligonucleotide probe, 5′-FAM-TCACCAACTGGGACGACATGGAGAAA-TAMRA-3′.

All quantitative values were expressed as means ± SD. The significance of differences for the numerical traits between model and control rats was tested using the *t*-test. The significance of differences of the quantitative values among the different concentrations of glucose, mannitol, insulin, and glucagons was analyzed by one-factor analysis of variance or rank sum test. The Student–Newman–Keuls (SNK) test was used for further comparison. Two-sided $p < 0.05$ values were considered to be statistically significant.

RESULTS

The table shows the characteristics of the model rats. Compared with the control rats, the model rats had more weight increase, higher levels of glucose and triglycerides, showing hypercoagulable status, and therefore some features of MS.

Characteristics of model rats compared with control rats (means ± SD)

Parameter	Control rats ($n = 11$)	Model rats ($n = 12$)
Weight increase, %	8.30 ± 5.0	14.30 ± 4.2*
Glucose, mM	8.10 ± 1.7	10.30 ± 1.9*
Triglycerides, mM	0.48 ± 0.21	1.39 ± 0.33*
APTT, sec	24.80 ± 4.5	20.80 ± 4.4*
PT, sec	13.40 ± 2.0	10.00 ± 1.1*
PAI-1, IU/ml	0.41 ± 0.07	0.50 ± 0.09*

* $p < 0.05$ compared with control rats.

The mixture of differentially expressed fragments from the rat hepatic subtracted cDNA libraries was used to screen a human hepatic library. After three screenings (Fig. 1), 68 positive clones were found. Twenty randomly identified full-length cDNAs were sequenced. One of the full-length cDNAs identified has not been reported before, and we temporarily named it as metabolic syndrome-associated gene, *MSAG*. The *MSAG* cDNA

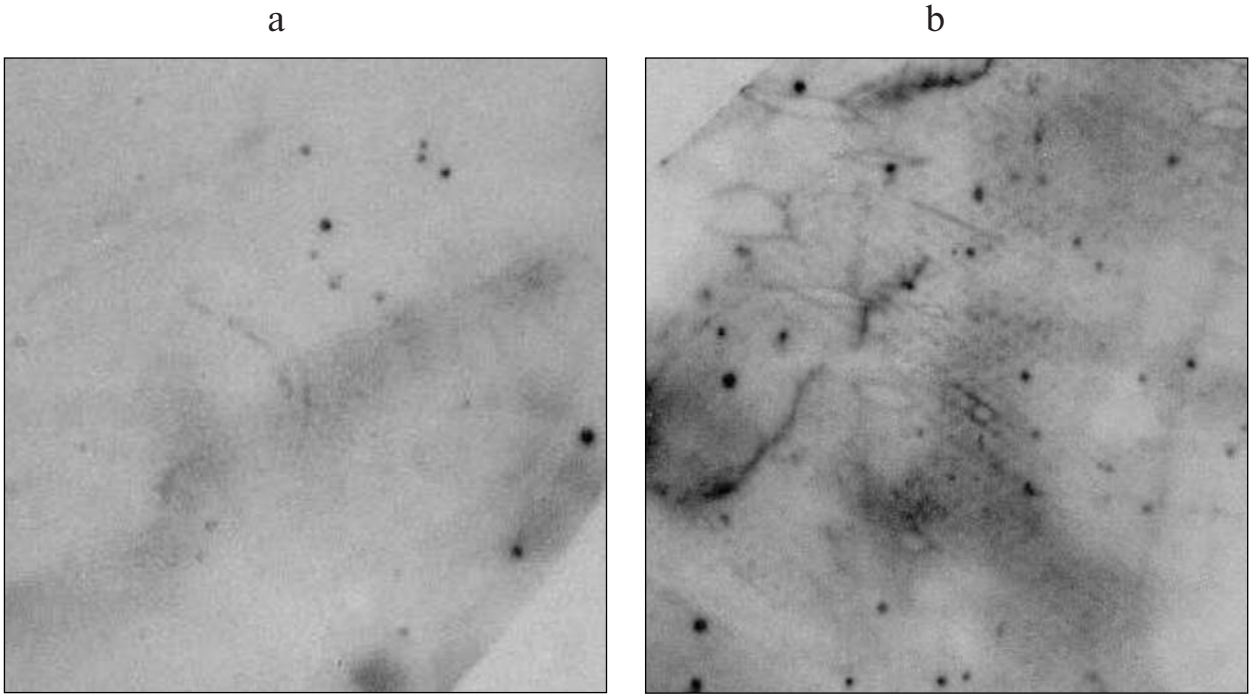


Fig. 1. Screening of a human hepatic cDNA library by the mixture of differentially expressed fragments amplified by PCR from the positive cDNA clones of the rat hepatic subtracted cDNA libraries: a) second screening; b) third screening.

```
a     MAASSSSSSAGGVSGSSVTGSGFSVSDLAPPRKALFTYPKGAGEMLEDGSERFLCESVFSYQVASTLKQVKHDQQVARMEKLAGLVEELEADEWRFKPIEQLLGFTPSSG 110
b     MAASSSSSSAGGVSGSSVTGSGFSVSDLAPPRKALFTYPKGAGEMLEDGSERFLCESVFSYQVASTLKQVKHDQQVARMEKLAGLVEELEADEWRFKPIEQLLGFTPSSG 110
c     MAASSSSSSAGGVSGSSVTGSGFSVSDLAPPRKALFTYPKGAGEMLEDGSERFLCESVFSYQVASTLKQVKHDQQVARMEKLAGLVEELEADEWRFKPIEQLLGFTPSSG 110
d     MAASSSSSSAGGVSGSSVTGSGFSVSDLAPPRKALFTYPKGAGEMLEDGSERFLCESVFSYQVASTLKQVKHDQQVARMEKLAGLVEELEADEWRFKPIEQLLGFTPSSG 110
e     MAASSSSSSAGGVSGSSVTGSGFSVSDLAPPRKALFTYPKGAGEMLEDGSERFLCESVFSYQVASTLKQVKHDQQVARMEKLAGLVEELEADEWRFKPIEQLLGFTPSSG 110
f     MAASSSSSSAGGVSGSSVTGSGFSVSDLAPPRKALFTYPKGAGEMLEDGSERFLCESVFSYQVASTLKQVKHDQQVARMEKLAGLVEELEADEWRFKPIEQLLGFTPSSG 110
g     MAASSSSSSAGGVSGSSVTGSGFSVSDLAPPRKALFTYPKGAGEMLEDGSERFLCESVFSYQVASTLKQVKHDQQVARMEKLAGLVEELEADEWRFKPIEQLLGFTPSSG 110
h     MAASSSSSSAGGVSGSSVTGSGFSVSDLAPPRKALFTYPKGAGEMLEDGSERFLCESVFSYQVASTLKQVKHDQQVARMEKLASLVEELEADEWRFKPIEQLLGFTPSSG 110
i     MAASSSSSSAGGVSGSSVAGSGFSVSDLAPPRKALFTYPKGAGEMLEDGSERFLCESVFSYQVASTLKQVKHDQQVARMEKLAGLVEELEADEWRFKPIEQLLGFTPSSG 110
j     MAASSSSSSAGGVSGSSVPGSGFSVSDLAPPRKALFTYPKGAGETLEDGSERFLCESVFSYQVASTLKQVKHDQQVARMEKLAGLVEELEADEWRFKPIEQLLGFTPSSG 110
ruler 1……10…….20……..30……. 40……50……60……...70……..80……90……...100……110
```

Fig. 2. Multiple mammal alignment of the amino acid sequence of MSAGs. The numbering was based upon the amino acid sequence of MSAG. The residues in each column were designated as white or black according to the consensus character assigned to that column: a) MSAG; b) human; c) mouse; d) Bornean orangutan; e) rhesus monkey; f) horse; g) dog; h) pig; i) cow; j) rat.

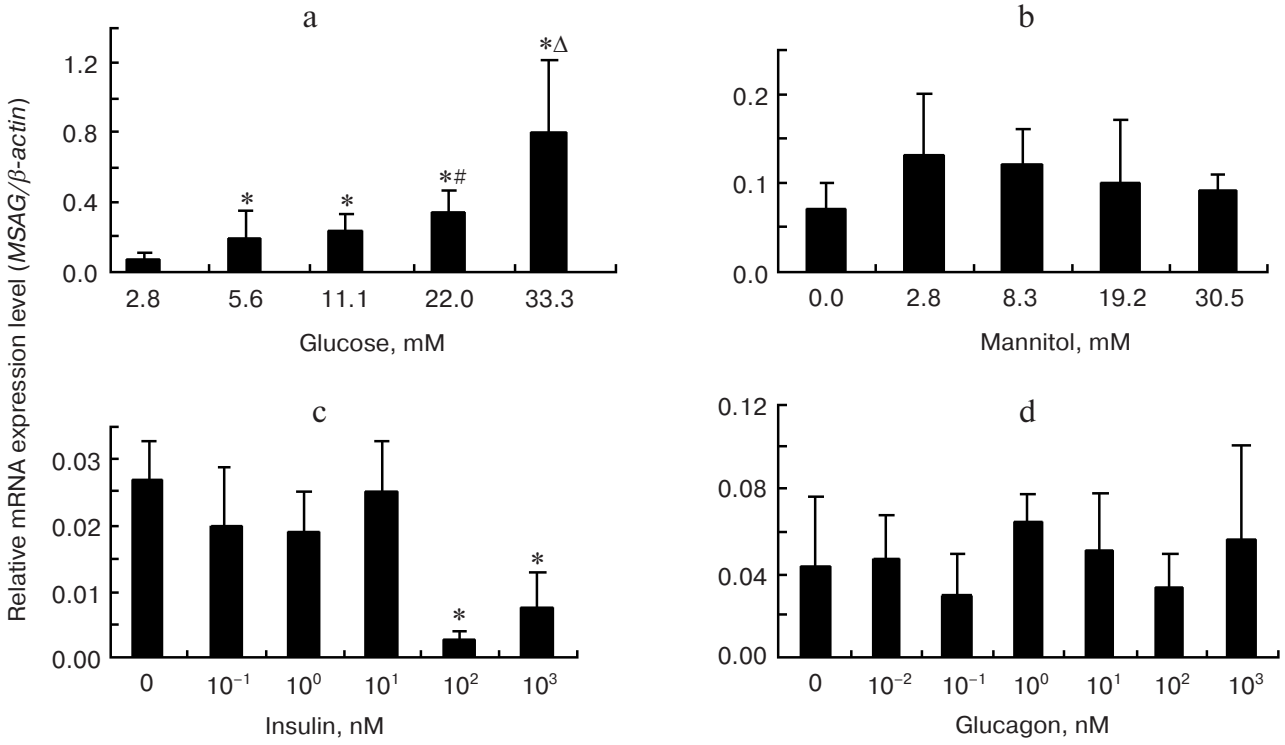


Fig. 3. Effect of glucose (a), mannitol (b), insulin (c), and glucagon (d) on the *MSAG* mRNA to *β-actin* mRNA ratio in HepG2 cells. Values are expressed as mean ± SD of four independent experiments and triplicates in each experiment. a) * $p < 0.05$ compared with that at the concentration of 2.8 mM; # $p < 0.05$ and Δ $p < 0.001$ compared with those at other concentrations; c) * $p < 0.05$ compared with those at the concentrations of 0, 0.1, 1, and 10 nM.

sequence was submitted to GenBank as a novel gene with Accession No. EU552924.

MSAG encodes a polypeptide of 110 amino acids with a theoretical molecular weight of 11667.04 and an isoelectric point of 4.91. This protein has no signal peptide. It has four N-myristoylation sites at 11-16 (GGvsGS), 12-17 (GVsgSS), 15-20 (GSsvTG), and 20-25 (GSgfSV), one casein kinase II phosphorylation site at 24-27 (SvsD), and two protein kinase C phosphorylation sites at 50-52 (SeR) and 66-68 (TlK). These results suggest that this protein is unlikely to be a secretory protein and may play a role in some signaling pathways. Figure 2 shows amino acid sequence comparison of known MSAGs from different mammals.

To test the effects of glucose, insulin, and glucagon on *MSAG* expression, the mRNA copies of *MSAG* in HepG2 cells were measured and normalized with the copies of the housekeeping gene of *β-actin*. As shown in Fig. 3a, the mRNA ratio of *MSAG* to *β-actin* increased when the cells were incubated with glucose from below physiological concentration (2.8 mM) to physiological concentration (5.6 mM). Increased concentration of glucose from 5.6 to 11.1 mM did not significantly change the mRNA ratio. When glucose was further increased from

11.1 to 22 and 33.3 mM, the mRNA ratio was increased significantly. No effects were observed of mannitol as a control of osmotic pressure on the expression of *MSAG* in HepG2 cells (Fig. 3b). In addition, insulin with its concentration varying from 0.1 to 1 and 10 nM under physiological conditions had no effects on the mRNA ratio (Fig. 3c). Further increase of insulin from 10 to 100 nM significantly lowered the mRNA ratio. However, the effect of insulin at concentrations between 100 and 1000 nM on the mRNA ratio was not significantly different. No significant effects were found of glucagon on the mRNA ratio (Fig. 3d).

DISCUSSION

Changes of the level and the pattern of gene expression are one of the main driving forces of biological processes in living organisms, and their disorders may result in pathological alterations [19]. It is important to identify these changes in order to understand the molecular mechanism of related diseases. The pathogenesis of MS is complex and has not been well elucidated yet. A rat model has been used in our laboratory to study the derangements of metabolisms of glucose and triglycerides because the model animals showed the phenotypes of hypertriglyceridemia, an increase of body weight, a higher level of plasma glucose, and a lower level of HDL-C [20].

To explore the function of *MSAG* and confirm the association of *MSAG* with the phenotypes of MS, the effects of glucose, insulin, and glucagon on the mRNA levels of *MSAG* in HepG2 cells, a developed human hepatoma cell line possessing shapes and functions similar to freshly isolated hepatocytes, were tested [21]. When the cells were cultured with a physiological concentration of glucose and without any other stimuli, the mRNA ratio of *MSAG* to *β-actin* was found to be from 0.027 to 0.19 (Fig. 3), indicating that *MSAG* has low transcription activity compared to the housekeeping gene of *β-actin*. Given that 2.8 mM is far less than the necessary concentration of glucose required for normal growth and proliferation of cells, these results indicate that *MSAG* is up regulated by glucose but down-regulated by insulin at higher than physiological concentrations. This suggests that this novel gene might be involved in the development of MS.

The decrease of *MSAG* mRNA in the HepG2 cells cultured with high concentrations of insulin was probably due to activation of the mitogen-activated protein kinase (MAPK) cascade. HepG2 cells express insulin receptor [22]. This receptor is a receptor tyrosine kinase (RTK) that uses docking proteins, such as insulin receptor substrates (IRS) 1-6, to subsequently activate the MAPK cascade. It has been previously reported that this process is involved in the regulation of gene expression [23]. No significant change of *MSAG* mRNA expression was observed in cells treated with insulin at 100 and 1000 nM, which could be the result of binding saturation of insulin receptor or an insulin-stimulated down-regulation of the insulin receptor by high concentrations of insulin [24]. The response of *MSAG* mRNA to glucose was probably induced by inhibition of AMP-activated protein kinase (AMPK) or activation of protein kinase C (PKC). Zang et al. [25] reported that exposure of HepG2 cells to elevated glucose (30 mM) for 24 h decreased AMPK phosphorylation and inhibited AMPK, which was involved in the regulation of the expression of some genes in cells [26]. Raile et al. [27] reported that the gene expression of IGF-I receptor (*Igf1r*), insulin receptor (*Insr*), insulin receptor-related receptor (*Insrr*), and the metabolic glycolysis gene liver-type pyruvate kinase (*Pklr*) was increased in rat islets exposed to high glucose (25 mM) compared with intermediate (6.2 mM) or low glucose concentration (1.6 mM). AMPK might be involved in the regulation of *Igf1r*, *Insr*, and *Insrr* expression. It is known that high glucose increases the level of diacylglycerol (DAG) in cells via *de novo* synthetic pathways, with glycerol-3-phosphate, an intermediate of glycolysis. DAG newly generated from *de novo* synthesis is distributed to intracellular DAG pools. Increased cellular DAG leads to activation of specific PKC isoforms [28]. Activated PKC mediated by high glucose also has been shown to regulate the gene expression of many proteins at the mRNA level [29, 30].

In addition, insulin also can activate PKC through DAG pathway and produce a series of biological effects via protein phosphorylation. RTK of insulin activates phospholipase C (PLC), which hydrolyzes phosphatidylinositol biphosphate (PIP2) and produces DAG. This product subsequently induces an increase in phosphorylation and translocation of specific PKC isoforms [31]. However, the PKC isoforms activated by glucose and insulin are not identical [31, 32] and this could be an explanation for the different changes of the expression of *MSAG* mRNA induced by glucose and insulin. Overactivation of PKC isozymes could lead to insulin resistance (IR). PKC has been demonstrated to phosphorylate serine/threonines in both the insulin receptor and IRS-1 [33], leading to the decrease in the ability of insulin to stimulate glucose transport and its metabolic effects. Another mechanism of IR induced by the activation of PKC is probably via the increase of oxidative stress and the activation of IκB kinase (IKK) and/or the nuclear factor (NF)-κB pathway. It has been shown that hyperglycemia can increase oxidative stress and activate NFκB, mediated by PKC [34, 35]. In general, the principal pathophysiology of MS is an insulin resistance, which, at the cellular level, is the result of changes in the signaling pathway triggered by the activation of insulin receptor. One of the key changes is the alteration of phosphorylation of intermediate proteins. MSAG protein has two potential PKC phosphorylation sites. Once the PKC is activated, PKC phosphorylation sites of MSAG protein could be phosphorylated. Furthermore, MSAG protein has one potential casein

kinase II phosphorylation site. Casein kinase II (CKII) is a ubiquitous serine/threonine protein kinase found in eukaryotic cells. Insulin can rapidly increase the activity of CKII. This protein kinase may act on glycogen synthase by direct phosphorylation of the enzyme and through phosphorylation of the inhibitor 2 of protein phosphatase 1, which is a prerequisite for or potentiates the subsequent action of glycogen synthase kinase 3 (GSK3) on these substrates [36]. CKII may alter the DNA binding of some substrates by phosphorylation *in vitro*, which include several steroid hormone receptors. MSAG protein might be an intermediate protein in the signaling pathway, phosphorylated by PKC and CKII. By this process, MSAG protein might deliver signals to subsequent intermediate(s) and influence metabolisms of glucose and lipids.

In summary, a novel gene, *MSAG*, has been identified, which may be associated with derangements of the metabolisms of glucose and triglycerides. Although the function of *MSAG* and its possible roles in the development of MS have not been elucidated yet and are currently under investigation in our laboratory, identification of this novel gene may provide new ways for the elucidation of the mechanism underlying MS and thus to possible better therapy of MS.

The authors are grateful to Dr. Yue Zou at East Tennessee State University for his significant advice and consultation on the manuscript.

This study was supported by the National Natural Science Foundation of China (30571026) and the Program of Sichuan Province for International Cooperation and Exchanges of Sciences and Technology (2008HH0008). Ding Zhi Fang is the recipient of the grants.

REFERENCES

1. Reaven, G. M. (1988) *Diabetes*, **37**, 1595-1607.
2. Festa, A., D'Agostino, R. J., Howard, G., Mykkanen, L., Tracy, R. P., and Haffner, S. M. (2000) *Circulation*, **102**, 42-47.
3. Messerli, A. W., Seshadri, N., Pearce, G. L., Sachar, R., Hoogwerf, B. J., and Sprecher, D. L. (2003) *Am. J. Cardiol.*, **92**, 610-612.
4. Vaisanen, S., Baumstark, M. W., Penttila, I., Bouchard, C., Halonen, P., Rankinen, T., Berg, A., and Rauramaa, R. (1997) *Thromb. Haemost.*, **78**, 1495-1499.
5. Lorenzo, C., Okoloise, M., Williams, K., Stern, M. P., and Haffner, S. M. (2003) *Diabetes Care*, **26**, 3153-3159.
6. Ninomiya, J. K., L'Italien, G., Criqui, M. H., Whyte, J. L., Gamst, A., and Chen, R. S. (2004) *Circulation*, **109**, 42-46.
7. Bonora, E., Kiechl, S., Willeit, J., Oberhollenzer, F., Egger, G., Bonadonna, R. C., and Muggeo, M. (2003) *Diabetes Care*, **26**, 1251-1257.
8. Owen, O. E., Kalhan, S. C., and Hanson, R. W. (2002) *J. Biol. Chem.*, **277**, 30409-30412.
9. Kane, J. P. (1983) *Annu. Rev. Physiol.*, **45**, 637-650.
10. Carew, T. E., Pittman, R. C., and Steinberg, D. J. (1982) *Biol. Chem.*, **257**, 8001-8008.
11. *Third Report of NCEP* (2001) NIH Publication No. 13670.
12. Corella, D., and Ordovas, J. M. (2004) *Curr. Atheroscler. Rep.*, **6**, 186-196.
13. Balkau, B., Charles, M. A., Drivsholm, T., Borch-Johnsen, K., Wareham, N., Yudkin, J. S., Morris, R., Zavaroni, I., van Dam, R., Feskins, E., Gabriel, R., Diet, M., Nilsson, P., and Hedblad, B. (2002) *Diabetes Metab.*, **28**, 364-376.
14. Isomaa, B., Almgren, P., Tuomi, T., Forsen, B., Lahti, K., Nissen, M., Taskinen, M. R., and Groop, L. (2001) *Diabetes Care*, **24**, 683-689.
15. Meigs, J. B., Wilson, P. W., Nathan, D. M., D'Agostino, R. B., Williams, K., and Haffner, S. M. (2003) *Diabetes*, **52**, 2160-2167.
16. Ravussin, E., Valencia, M. E., Esparza, J., Bennett, P. H., and Schulz, L. O. (1994) *Diabetes Care*, **17**, 1067-1074.
17. Kolovou, G. D., Anagnostopoulou, K. K., and Cokkinos, D. V. (2005) *Postgrad. Med. J.*, **81**, 358-366.
18. Shen, T., Bai, H., Liu, B. W., and Zhang, Z. H. (2002) *Chin. J. Arterioscler.*, **10**, 199-202.
19. Lasko, D., Cavenee, W., and Nordenskjold, M. (1991) *Annu. Res. Genet.*, **25**, 281.
20. Zhao, Y. H., Bai, H., Liu, B. W., Shen, T., Fang, D. Z., and Liu, Y. (2005) *Biochem. Biophys. Res. Commun.*, **334**, 162-169.
21. Podskalny, J. M., Takeda, S., Silverman, R. E., Tran, D., Carpentier, J. L., Orci, L., and Gorden, P. (1985) *Eur. J. Biochem.*, **150**, 401-407.
22. Moxham, C. P., Duronio, V., and Jacobs, S. (1989) *J. Biol. Chem.*, **264**, 13238-13244.
23. Pawelczyk, T., Sakowicz, M., Podgorska, M., and Szczepanska-Konkel, M. (2003) *Exp. Cell Res.*, **286**, 152-163.
24. Russell, D. S., Gherzi, R., Johnson, E. L., Chou, C. K., and Rosen, O. M. (1987) *J. Biol. Chem.*, **262**, 11833-11840.
25. Zang, M., Zuccollo, A., Hou, X., Nagata, D., Walsh, K., Herscovitz, H., Brecher, P., Ruderman, N. B., and Cohen, R. A. (2004) *J. Biol. Chem.*, **279**, 47898-47905.
26. Diraison, F., Motakis, E., Parton, L. E., Nason, G. P., Leclerc, I., and Rutter, G. A. (2004) *Diabetes*, **53**, S84-S91.
27. Raile, K., Klammt, J., Laue, S., Garten, A., Bluher, M., Kralisch, S., Kloting, N., and Kiess, W. (2005) *Diabetologia*, **48**, 1798-1809.
28. Kanehara, H., Suzuki, J., Zenimaru, Y., Takahashi, S., Oida, K., Shen, W. J., Kraemer, F. B., and Miyamori, I. (2004) *Diabetes Res. Clin. Pract.*, **65**, 209-215.
29. King, G. L., and Brownlee, M. (1996) *Endocrinol. Metab. Clin. North Am.*, **25**, 255-270.
30. Williams, B. (1995) *J. Hypertens.*, **13**, 477-486.
31. Wright, D. C., Fick, C. A., Olesen, J. B., and Craig, B. W. (2003) *Life Sci.*, **73**, 61-71.
32. Das Evcimen, N., and King, G. L. (2007) *Pharmacol. Res.*, **55**, 498-510.
33. Ravichandran, L. V., Esposito, D. L., Chen, J., and Quon, M. J. (2001) *J. Biol. Chem.*, **276**, 3543-3549.
34. Inoguchi, T., Li, P., Umeda, F., Yu, H. Y., Kakimoto, M., Imamura, M., Aoki, T., Etoh, T., Hashimoto, T., Naruse, M., Sano, H., Utsumi, H., and Nawata, H. (2000) *Diabetes*, **49**, 1939-1945.
35. Coudronniere, N., Villalba, M., Englund, N., and Altman, A. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 3394-3399.
36. Fiol, C. J., Wang, A., Roeske, R. W., and Roach, P. J. (1990) *J. Biol. Chem.*, **265**, 6061-6065.